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CERTAIN ASPECTS OF THE EPIDEMIOLOGY OF LEPTOSPIROSIS IN JAMAICA

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CERTAIN ASPECTS OF THE EPIDEMIOLOGY OF LEPTOSPIROSIS IN JAMAICA

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Leptospirosis is an acute infectious illness of world-wide scope. Although basically a zoonosis, man is often an incidental and sometimes a tragic victim.

In 1886 Adolf Weil (1), Professor of Medicine at Heidelberg, described four cases of severe acute illness characterized by marked fever, hepatosplenomegally, jaundice, renal involvement, nervous system symptoms, followed by rapid recovery. These cases he differentiated clinically from the known entities of infectious hepatitis, primary nephritis, a combination of infectious hepatitis and primary nephritis, yellow atrophy of the liver, septicemia, recurrent fever, and bilious typhoid fever.

Although Landouzy (2) in Paris had described the same disease in 1883 the condition became termed Weil's disease by German physicians. Interestingly, Landouzy had associated the disease with work in sewers and he suggested that a miasma from the sewage caused it.

During the next few decades numerous cases were reported in Southwest England, Germany, Adaman Islands (in the Indian Ocean), and around the Mediterranean Region (3).

Inada et. al. (4) (published in 1916) described Weil's disease in Japan as an epidemic and endemic disease characterized by conjunctival congestion, muscular pain, fever, jaundice, hemorrhagic diathesis, and albuminuria. They called it "febrile jaundice" and Odan-eki" or icteric pestilence." Clinically it compared closely with European Weil's but tended to have a more endemic quality in Japan than that described in Europe. They differentiated it from bilious typhoid, which it resembles, yellow fever, and infectious hepatitis.

Having vainly sought bacteria in patients' blood, urine, and feces, they performed various animal injection studies with patients' blood. In 1914 they found that guinea pigs developed albuminuria, conjunctival congestion, jaundice, and hemorrhages following inoculation of blood taken from the patient within the first seven days of illness. They managed to transmit the disease to other guinea pigs in intraperitoneal, subcutaneous injection and oral feeding of heart blood or ground liver. There were consistent findings of large numbers of spirochetes in autopsied livers. Guinea pig injection with blood of healthy subjects and infectious hepatitis patients gave no such pathologic changes.

The spirochetal etiology of Weil's was confirmed by demonstrating morphologically identical spirochetes in the blood of six patients, the intestinal wall of one autopsy case, and the adrenal glands of another. Furthermore, these workers discovered a spirochetocidal substance in the sera of convalescent patients. This they assumed was the reason for decreased numbers of organisms in autopsy tissues from patients dying in later stages of the disease.

They were able to culture and repeatedly subculture the organisms, which they called Spirochaeta icterohaemorrhagiae, employing the method Noguchi (5) had used for culturing the spirochetes of recurrent fever. Subcultured organisms into at least the third generation were infectious for guinea pigs, producing the usual pathologic picture. Ideal temperature for pure culture was 22-25°C (room temperature). Spirochetes died in several days at 37°C and no growth was obtained below 15°C.

The organisms were passable through Berkefeld cylindrical filters

which would not permit passage of ordinary bacteria.

The group investigated experimental infection in animals. Earlier literature suggestions as to natural modes of infection had included: via the alimentary tract, through the skin, and by insect bite. Inada and his group produced guinea pig infection through macroscopically healthy (but shaved) abdominal skin as well as abraded skin. Even with an alcohol wash of the skin 5 minutes following contact with infected liver emulsion infection was produced. Infection through unbroken skin had a longer incubation than abraded skin (9-10 days vs. 7-8 days respectively). Infection was established by feeding liver homogenates containing spirochetes by mouth and also by an enema of the same homogenate.

With regard to mode of excretion of the organisms the group noted: 1) Spirochetes in patients' urinary sediment by darkfield exam and guinea pig inoculation while patients were in the acute phase of the disease. 2) Spirochetes by the same methods in the urinary sediment of experimental animals as early as the 6th and as late as the 38th day post inoculation. They were most numerous on about the 15th day -- long after antibody (see below) was demonstrable. 3) Spirochetes by guinea pig injection in the feces and bile of experimental animals. 4) Infectious spirochetes present on one occasion in the stools of an infected patient and in the bloody sputem of another.

The group was first to demonstrate protective antibody in this disease (Pfeiffer's reaction):

1) Infected liver emulsion plus immune serum injected intraperitoneally into guinea pigs yielded no spirochetes in the blood in one

half hour by darkfield and usually no infection. Appropriate controls with normal serum or saline resulted in darkfield-visible spirochetes even as late as 2 hours and always produced infection.

2) Acutely infected guinea pigs when given immune serum had no demonstrable organisms in the blood by one half hour and only degenerate forms in the liver within a few hours by silver stain. The organisms were still found in the kidneys and adrenals however,

3) Sera from patients were effective in Pfeiffer's method after 14 to 15 days but never before the 11th day of illness. Sera worked in this phenomenon up to 5 1/2 years post infection, which was the longest tested.

The group noted some of the epidemiology of Weil's disease as it appeared in Japanese coal mines. (The disease had been recognized as an occupational hazard of mining in the British Isles and Flanders.)

1) The disease was more common among workers in some parts of the mines than others.

2) Numerous cases did not arise in the same living quarters even when the disease "originated" there.

3) Clerks working outside the mines did not acquire the disease.

4) Many cases were seen in wet mines but few in dry ones.

5) Numerous cases were occurring among miners who worked in certain parts of one mine investigated. When the accumulated water was pumped out (at the suggestion of one of the investigators) no further cases occurred.

6) The finding that the disease was common among two particular

nearby communities yet uncommon in a third community directly across a river from them suggested against insect transmission.

7) There were instances where two or three family members were affected simultaneously. The investigators could offer no explanation for the mechanism of transmission here. (They did not suggest a common source of drinking or bathing water.)

Some of these workers, Ido et. al. (6) in 1916 attempted active and passive immunization in man and animals with equivocal results.

In 1915 Miyajima (7) reported finding spirochetes resembling *S. icterohaemorrhagiae* in field mice kidneys while investigating tsutsugamushi fever. Ido et. al. (6) the following year also made this finding in house and roof rats. In 1916 Miyajima (7) made guinea pig injections of spirochetes in a mouse kidney and produced fever and hemorrhage and, after several generations, jaundice. Immune serum to *S. icterohaemorrhgiae* was capable of destroying these mouse spirochetes. In 1917 Ido et. al. (6) reported finding virulent *S. icterohemorrhagiae* by guinea pig inoculation in the kidneys of 86 house and ditch rats thus demonstrating en masse what has turned out to be the major carrier and vector of pathogenic leptospires.

The spirochetal findings of the Japanese workers were confirmed during the War in Europe when hundreds of cases occurred among British and other troops (8,9,10,11).

The first of what are now known as Leptospires was isolated in 1913 from a fresh water pond near Boston, Massachusetts, by Wolbach and Binger (12). Having passed pond water through a Berkefeld "V" filter,

they discovered cloudiness in the filtrate after one month incubation. A pure culture of a spirochete was found by darkfield examination. It did not survive subculture into various media which had previously supported free-living spirochetes, and it was not tested for pathogenicity in animals. The organism was extremely similar in morphology and motility to the *Spirochaeta biflexa* of Wolbach and Binger. Both passed the Berkefeld filter. Noguchi suggested that they comprise a new and distinct genus which he named Leptospira (- fine and - coil), to be included within the order *Spirochaetales* with *Cristispira*, *Saprospira*, *Spirochaeta*, *Spironema*, and *Treponema*.

Leptospira as a generic name has come into general acceptance since 1918. *L. icterohaemorrhagiae* became the type species or "serotype" because *L. biflexa* had not survived. Since this time numerous saprophytic and pathogenic serotypes (for the only known distinction among "species" is serologic) have been isolated and identifed. Investigators have found the organisms in Europe, Australia, the U.S.A., Japan, Indonesia, and elsewhere in tap water, running and stagnant water, mud, in almost every conceivable mammal and in great numbers of human patients. There are currently over three dozen known pathogenic serotypes and sub-serotypes and numerous saprophytic leptospires.

Manifestations of leptospiral infection in animals range from asymptomatic to fatal.

In man the infection can also take many forms. The different serotypes lead to somewhat different clinical syndromes but there is much

overlap. Classic Weil's disease is associated with *L. icterhemorrhagiae* (3,23,24) but at times also with *L. bataviae* (3,23), *L. kremastos* (as shown in this report), and others (3).

After an incubation of 4 to 19 days (average 7-14) the disease begins acutely, often with shaking chills and usually with a marked fever. The temperature can reach 102 to 104°F, and is maintained for over a week before falling by lysis. Severe anorexia, prostration, and headache are customary. Frequent complaints are nausea, vomiting, and diarrhea with abdominal tenderness. Myalgia and muscle tenderness, often exquisite, and arthralgia are seen in a large but variable number of the cases. Moderate to high leukocytosis with a shift to the left (as contrasted with typhoid) is typical.

Bulbar and palpebral conjunctival injection is quite common, developing early. Other eye manifestations, conjunctival hemorrhage, retinal hemorrhage, and some neuro-opthalmologic dysfunction are reported in the actue phase but usually resulve without sequelae. Optic atrophy is rare.

Pharyngitis with cough is common. A marked leptosprial pneumonitis can be part of the acute disease. Generalized lymphadenopathy is fairly typical with splenomegally less so.

Jaundice, which may be prolonged, usually appears at the end of the first week simultaneous with defervescense and may be the patient's chief complaint. Hepatomegally and disturbed liver functions are customary in icteric and even in some anicteric cases. Jaundice is usually a part of the severe and fatal cases. If the patient lives, the hepatitis customarily clears without sequelae.

Some renal involvement is characteristic of the disease. A nephritis develops with anuria or oliguria and heavy proteinuria in the severe cases. At the very least some transient proteinuria is usually seen. Renal decompensation with uremia and electrolyte imbalance can be the cause of death. Premanent renal lesions are very rare.

Hemorrhagic manifestations may be part of the clinical picture with cutaneous petechiae, and gastrointestinal hemorrhage. Severe peripheral vascular decompensation or myocarditis and pericarditis may develop. Acute adrenal insufficiency can occur in a Waterhouse-Friedrickson fashion.

Central nervous system involvement, notably meningitis, with meningeal signs, elevated cerebrospinal fluid pressure, and lymphocytic pleocytosis may be present. The "aseptic" meningitis clears completely in the convalescent period but some temporary residual neuritis has occurred.

Death rates of up to 30 per cent have been reported in various series (3,23,24). The commonest cause is acute renal failure and uremia. However in the earliest stages of the disease vascular collapse or gastrointestinal hemorrhage can be the chief cause. Pneumonia and less commonly myo- and pericarditis may certainly complicate the course.

Less severe manifestations of leptospiral infection can take many forms, usually aspects of the severe syndrome. Certain serotypes of leptospires are associated with these milder cases but the most virulent species -- L. icterohemorrhagiae, L. bataviae -- can also produce the milder disease pictures.

McCrumb and group (25) studied 244 confirmed cases among military

and civilian personnel in Malaya in 1954 and 1955. From these patients 90 leptospiral isolations were made involving 12 serotypes (10 isolations of L. bataviae, 6 L. icterohemorrhagiae). For the overall group the death rate was 0.8 per cent. Typical clinical features were acute fever, headache, gastrointestinal symptoms, and conjunctival injection. About three fourths of the group had myalgia, respiratory symptoms, and fleeting proteinemia. About half had retrobulbar pain and abdominal tenderness. Less common were hemorrhagic manifestations -- hemotysis, rash -- and meningeal signs. Jaundice and hepatomegally were quite uncommon as were anuria and oliguria. One man who recovered from a case of leptospirosis (L. schüffneri), became infected four months later with another serotype (L. bataviae). The two deaths in the series were from L. bataviae and L. australis,B, respectively.

In review of the literature Stockard and Woodward (23) briefly characterized the disease patterns associated with the various leptospires. L. icterohemorrhagiae and L. bataviae produce variable clinical pictures ranging from classical Weil's to very mild cases. L. canicola is different from these two only in that it rarely produces jaundice and more commonly gives a lymphocytic meningitis.

L. pomona is responsible for "swineherd's disease" in Australia and Europe and some outbreaks in Alabama. The clinical course is mild with fever five to seven days. A high rate of lymptocytic meningitis is common, but hemorrhagic phenomena, cardiovascular, hepatic and renal disorders are unusual. Beeson (26) has implicated leptospires, particularly L. pomona, with a benign, lymphocytic "asceptic" meningitis in

the United States.

L. autumnalis, first isolated in Japan, was responsible for an outbreak of acute illness among soldiers at Fort Bragg, North Carolina in the early 1940's. "Fort Bragg fever" ran a short febrile period with moderate prostration, splenomegally, and rash over the pretibial aspects of the legs.

"Swamp fever" -- Schlammfieber" -- is quite common throughout Central and Eastern Europe and is caused by L. grippotyphosa. A benign one to two week course with rare jaundice or miningitis features fever, severe headache, muscle pain, gastrointestinal dysfunction, and a variety of rashes (3).

L. hebdomadis (3) is a cause of "seven-day fever" in the Orient with a benigh short course including fever, prostration, conjunctival injection, muscle pain, and gastrointestinal upset. L. kremastos and L. sejroe are both serotypes in the L. hebdomadis serogroups and are both thought to produce mild ilness (3). This is of interest in view of the clinical picture found in Jamaica.

Since the time of the early Japanese workers considerable investigation has been carried out on the animal transmission and general epidemiology of leptospiral infection. Very active in this research has been Babudieri, who reviewed the state of knowledge in 1958 (15).

First he considered the carrier state as it applies to leptospires. In the natural course of animal infection, as in human infection, there is a period of nephritis during which leptospires are passed in the urine. In man and many animals the leptospiuria terminates with or

soon after the acute infection. The epidemiologic importance of these temporary shedders is limited. However, certain animals become carriers with a persistent leptospiruia, often for the entire life of the animal.

Various rodents, notably rats, are widely recognized as carriers but less is known about other animals. Man has never been shown to remain a carrier past early convalescence.

The epidemiologic significance among those animals which remain carriers depends on several factors:

1) The pH of the urine. Pathogenic leptospires are very sensitive to variations in pH. In Babudieri's studies the organisms survived at least six days under otherwise appropriate conditions in the range from pH 6.24 to 8.23. They survived 30 days from pH 6.35 to 7.96. Man and some animals usually have an acid urine, but it varies with diet. Zuelzer (16) reported urine of rats on a predominantly meat diet to be between pH 5.4 and 5.8. But if they subsisted on a primarily vegetable diet the pH ranged from 7 to 8. Babudieri found that dogs on a meat diet put out urine lethal to leptospires, but on a vegetable diet the urine was neutral to slightly alkaline. In general, herbivores produce neutral to alkaline urine and carnivores acid. However, acidity does not disinfect the urine of leptospires, which can tolerate an acid environment for short periods. If the urine is discharged into neutral or alkaline mud or water the organisms remain viable.

2) The physical environment of the animal carriers. Since most human infections take place indirectly -- via contact with mud, surface water, drinking water -- animals in moist non-acid regions are considerably more important than those in arid or acid areas. The wet season of the year permits a higher rate of transmission of leptospires among animals as well as to man.

3) The possibility or likelihood of direct or indirect contact with man.

Babudieri (15) reviewed the nature of pathology in the carrier state. As far as is known, infection involving transitory leptospiremia is necessary, with or without morbid manifestations. During the blood borne state it is possible to find spirochetes in all organs. In the kidneys they are present interstitially as well as in the vessels. Only relatively small numbers of leptospires, often coincidental with more or less extensive interstitial nephritis, penetrate the tubules and pass into the urine.

As circulating antibodies appear (7-10 days in humans) leptospires disappear from the blood and interstitial tissue and collect in the distal convoluted tubules. They appear as colonies, adhering to epithelial cells and can be found included in such cells usually with no pathologic manifestations. Masses of leptospires can become fairly large and tangled but seem not to obstruct the tubules. Organisms become detached from the masses and pass via the urine to the outside.

Babudieri considers this stage to be "external" parasitization.

Antibodies do not seem to contact the organisms and the organisms no longer act as antigens. In fact, in some animals antibody titers can fall below detectable levels during chronic colonization. As an equilibrium is reached, some species preferences become manifest (e.g. the rat, which is a notorious carrier of L. icterohemorrhagiae, appears not to be able to carry L. canicola (17)).

Babudieri further reported on the state of information on the ability of various animals to be carriers and the actual epidemiologic importance of the different animals. Several studies (15,18,19,20) have revealed the rare association of several ticks and mosquitoes with leptospires but insects are not considered of practical significance.

Amphibians, fish, and reptiles are not of demonstrated importance as carriers (15).

In a few instances certain wild and domestic birds have been found to be infected by leptospires or carry antibodies to the organisms (15,21,22). It would be interesting for obvious reasons if more birds, particularly migratory varieties, were found to be carriers.

However, while some lower animals may be susceptible, the major hosts for leptospires as well as the major carriers are mammals, especially rodents. In great numbers of studies throughout the world rats have been found to be the preëmanent carriers (3,15,24,27). Various mice have also been consistently implicated. Both of these animals live in varying intimacy with man, his foodstuffs, and domestic animals, in houses and barns, along waterways, and on agricultural lands. Most of the major leptospires are carried by at least one rodent. Surveys in

many parts of the world to find carriers (by culture of the kidneys) have revealed in endemic regions values as high as 60 per cent for rats (3,27) and rarely as high as 80 per cent for mice (3,27).

There may be a marked variation in the intensity of actual infection or parasitism within a population of potential carriers. Wide prevalence rates of carriers have been found among animals in neighboring areas. Alston and Broom (3) cite two examples: During an outbreak of swamp fever the infection rate with *L. grippotyphosa* among mice (*Microtus arvalis*) trapped in adjacent localities ranged from 5 to 80 per cent. Qualitatively similar results but with less divergence were found for infections with *L. icterhemorrhagiae* among brown rats (*Rattus norvegicus*) on a survey in rural South Whales.

As to mode of transmission of leptospires among carrier rodents several suggestions have been made. As yet definitive research is lacking. The importance of urine contaminated water is indicated by the finding in some studies of a much higher infection rate in rats trapped along water courses than in those trapped in fields distant from the streams (3). Transmission via contaminated food or direct contact with infective material has been proposed. The established finding of much higher infection rates among adult rather than young rats and some indication of the change occurring at the age of sexual maturity (3) have suggested a venereal transmission.

It is thought that a high infection rate among carrier rodents partly results from high density of the animals with frequency of contact with each other and with urine (3). Babudieri (15) cites some

interesting observations from Spain. In addition to wide variations in the numbers of rats infesting the different rice fields, there is considerable annual fluctuation in the carrier rate in any one place. Moreover, the investigating group, Altava, Barrera, and Marin (28), were able to quantatively predict the development of leptospirosis among field workers in the summer on the basis of the percentage of infected rats in the spring. With few rats infected, workers would show only the occasional case of disease, whereas with a high carrier rate an epidemic could be expected.

Among the rodents, Rattus norvegicus, the brown rat, is the most notorious. It is considered the chief carrier-host world-wide of L. icterohemorrgahiae and among the most important for L. bataviae (3). It is as well a know carrier of L. australis (3), L. ballum (24), and to a lesser degree of L. canicola (3).

R. norvegicus enjoys world-wide distribution as does L. icterohemorrhagiae. Thought to have first crossed the Volga from the East in 1720, the "Norway" rat reached the Baltic ports about 1729 (3). Then via ships the rat spread to all parts of the world. Originally from Asia, the brown rat is now located on the continents of North and South America, the British Isles, the Hawaiian Islands, Australia, Indonesia, and the West Indies. It is theorized that L. icterohemorrhagiae and the other leptospires came originally from Southeast Asia and spread like Plague with the rats by land then by sea to all corners of the world (3).

The various rats are ideal carriers. Able to harbor and shed most serotypes of septospires, and living in close proximity with man and

his animals, they become infected at early maturity with little or no morbidity or mortality and remain shedders throughout most of their lives (15).

Mice, also implicated in hosting many leptospires, have been found to be the major carriers for several serotypes -- *L. ballum*, *L. autumnalis*, *L. grippotyphosa* (3). They have been shown to carry the organisms for varying lengths of time, but unlike rats not for life.

Leptospires infect many domestic animals but the dog is considered of major epidemiologic importance to man. In many places the spread of the organisms among dogs is quite high. Ten to 20 per cent are most usual, but up to 60 per cent are found (15). The canine habit of sniffing where other dogs have urinated augments the more orthodox means of leptospiral transmission among animals.

With infection, dogs, especially the young, may become quite ill or even die. A chronic, progressive, and ultimarely fatal nephritis may follow the acute disease. Subsequent to infection dogs often, but not always, become carriers of *L. canicola* and less commonly *L. icterohemorrhagiae* (15) but not other types.

Cats have been rarely found to be carriers.

Often the pig is a leptospiral host and shedder and a source of infection to humans. While other types can be found, the two leptospires usually involved with swine are *L. pomona* and *L. hyos*. The former produces in these animals, particularly the young, an illness of variable severity, and can often induce abortion in sows. The latter serotype has an unknown morbid picture.

Following infection pigs may be carriers for periods up to a year, shedding great numbers of leptospires (3). Leptospiral infection is an occupational hazard of persons tending pigs. The so-called "swineherds disease" is primarily a benign aseptic-type meningitis picture with lymphocytic pleocytosis in the cerebrospinal fluid. In addition to more or less direct contact with swineherds, pig urine draining from sites has many times contaminated streams and rivers infecting those working or swimming in the water. After floods on land supporting pigs, epidemics have been observed (15).

Leptospirosis in cattle, aside from its serious economic consequences as a veterinary disease -- morbidity, mortality and spontaneous abortion, can lead to human infection. Following the acute phase the animals can remain shedders for limited durations -- an estimated average of 30 days in one study (15). Enormous quantities of the organisms are passed in the urine.

L. pomona and L. grippotyphosa in some places are the common serogroups involved with cattle. The former has not been shown to be of serious epidemiologic importance to man. But the latter, at least in central Europe, can be quite significant (15).

Sheep and goats become ill, sometimes fatally, with leptospirosis, and can become carriers for variable lengths of time. Horses and asses can contract clinical leptospirosis but have not been found to become true carriers.

Great numbers of wild animals have been found to be infected, yet with several exceptions either their biological response or habitat

preclude their relavance to human or domestic animal disease.

Bats, if found to be carriers of leptospires, would have obvious importance epidemiologically because of the migratory nature of many. They are very common animals, especially in the tropics, and live in variable proximity with man and his animals in the roofs of houses and barns. Though further research is warranted, when surveyed in regions where leptospires are found, bats have rarely yielded the organisms (3, 29). (In Indonesia L. schüffneri has been isolated in the animals (3)).

Until quite recently the mongoose has not been considered a common carrier or an important source of human infection. Babudieri (15), in 1958, in a compilation of animal-leptospiral associations listed under "other animals," Herpestes javanicus as a source for the isolation of L. javanica. Alston and Broom (3) in their classical work in 1958 mention H. javanicus as a carrier of subsidiary or unknown significance of L. javanica. No mention was made in either work of Herpestes auropunctatus, the mongoose imported from Southeast Asia to the West Indies, where it now thrives.

In 1963, Alexander et.al. (24) reported 11 isolations of leptospires from 55 mongooses (20 per cent) caught in Puerto Rico. Of these, 6 were identified: 4 L. icterohemorrhagiae, 2 L. djatzi, a new serotype in the bataviae serogroup. Serologic testing of the mongooses revealed 28 (50 per cent) positive. Twenty of these had predominant antibody titers to L. icterohemorrhagiae. Minette (27) in Hawaii, 1964, reported 18 isolations from 126 mongooses (14.3 per cent). He discovered 36 out of 126 (28.6 per cent) seropositive. The isolates were identified as

L's. icterohemorrhagiae, canicola, and sejroe (in the hebdomadis serogroup).

Inferences may be drawn on the significance of the mongoose as a carrier when it is considered that on many islands, of the West Indies particularly, the animals abound in the rural and agricultural regions.

Landouzy's 19th century work in Paris (13) in describing what came to be known as Weil's disease made noteof an association of the disease with sewer work. Inada and his group (4) in 1916 described the disease in coal miners and correlated it with the presence of water in the mines. By pumping out the water they were able to prevent further cases. In 1917 Ido and group (7) found L. icterohemorrhagiae in the kidneys of 86 mice and ditch rats. Since this time much of the general epidemiology of leptospirosis has been elucidated.

While considerable work remains in specific instances, it may be fairly presumed that in general the important animal carriers are known. Certain modes of transmission have been found when sought. The portals of entry of leptospires, as Inada et.al. (4) pointed out in 1916, are usually nasal, oral, and conjunctival mucous membranes, and the abraded skin. Unbroken skin is thought impenetrable to leptospires. The pH of the stomach makes it unlikely that the gastrointestinal tract is a portal (30).

Surface water, stagnant water, slow-moving streams, mud and moist soil where the pH is neutral to slightly alkaline and where the temperature is 22°C or above will allow the survival of leptospires for at least several weeks (15,30). Transmission of the organisms to man by

water is well documented.

Galton et.al. (30) reviewed numerous incidents. Swimming in contaminated water produced 35 non-icteric cases in an outbreak at Bushy Creek, Georgia, in 1940. It was found that refuse from slaughtered cattle had been dumped by local butchers 100 yards upstream from the swimming area. L. pomona was later ascribed as the offending organism. Fort Bragg fever, 1942-1944 (L. grippotyphosa) was often found in men who had been swimming in local ponds. Numerous other leptospiral outbreaks in the U.S. South and West and been traced to swimming pools, dammed up creeks, etc., to which animals had access. In one study the data showed significant correlation between attack rates and extent of exposure to water. Attack rates were consistantly higher among those giving a history of diving and immersing their heads.

Canals in the Netherlands have been known sources of infection for years. During World War II there were many cases of leptospirosis among pedestrians falling into canals during blackouts (3,31). (There are anecdotes about the aid of leptospires in Dutch fifth column activities. German occupation soldiers shoved into the canals might well expect to come down with the fever.) In England some of the early reported cases of Weil's disease, including the first proved case, were among men falling into the Thames. Great numbers of instances of the disease in the British Isles which were traced to swimming or accidental immersion in contaminated water were reviewed by Alston and Broom (3).

Water contact in agricultural settings -- rice paddies, sugar cane fields, grain fields -- all over the world are another well known source

of human infection. Galton et.al. (30) reviewed studies demonstrating leptospirosis in workers in rice fields in Italy, Spain, Japan, and other Pacific areas. These were regions where rats and mice abounded. The authors cite a study by Babudieri in the Po Valley which found 20 per cent of some 500 apparently healthy rice workers to have anti-leptospiral antibodies. *L. bataviae* was the antigen most often agglutinated. *L. grippotyphosa* was also found in various Peidmont rice fields (15). Sugar cane workers in North Queensland were demonstrated by Derrick and associates (32) to comprise over half of the known cases of leptospirosis. *L. australis A* was the predominant serotype, but 10 others were implicated. Then, of course, Central and Eastern European agricultural areas have endemic swamp fever, which is especially apparent after flooding leaves pools of mud in the fields (15).

Agricultural workers handling animals have been demonstrated in many places to be subject to leptospirosis. Galton et.al. (30) give numerous examples. In certain parts of Europe hog butchers, abbatoire workers and pig handlers commonly acquire swineherds' disease with *L. pomona* and *L. mitis*. Dairy farmers and pig breeders in New Zealand comprise the bulk of persons with leptospirosis. Numerous scattered cases of the disease in the U.S. have been traced to occupational contact with domestic animals -- livestock men, abbatoire workers, veterinarians, dog owners. The authors cite an instance where 9 members of one family acquired leptospirosis reputedly from the family dog. One-third of the reported cases in the U.S. in the early 1950's were in persons found to be in contact with contaminated swine or cattle.

A number of other occupations or situations involving direct or indirect contact with carrier animals or contaminated water are associated with an increased incidence of leptospirosis: dock workers, laboratory animal handlers, zoo keepers, soldiers on maneuvers, persons bitten by rats, mice, dogs, workers on grounds or in buildings infested with rats or mice (3).

LEPTOSPIROSIS IN JAMAICA

In the literature the earliest mention of leptospirosis in Jamaica, then a British West Indian Colony, appeared in 1952 in the first issue of the West Indian Medical Journal, published at the newly formed University College of the West Indies at Kingston. Guilbride (31) in a report on veterinary public health reviewed the contemporary state of knowledge on the disease. At that time no leptospirosis had been proved in animals, but it was suspected clinically in dogs. The author reported the outbreak of "confirmed" Weil's disease (with no mention of the means of confirmation) at Port Maria in 1948. Two persons were affected, both died. Other cases were suspected clinically.

The author had started an animal survey in 1948, continued it in 1951, but then had to abandon the project for lack of time, staff, and facilities. He had caught 38 rats, 8 mongooses, 8 mice, and 1 dog. These he examined for leptospires by histologic section of kidneys, liver, and adrenals, and by culture in Korthoff's medium of urine, liver, and kidneys. Four of the rats (3 from Kingston, 1 from Hope) "were considered to be harbouring leptospires as determined by liver and kidney

smears and culture." One spirochete was seen in one liver section of a mongoose. Guilbride was not able to follow up on his findings and the pathogenicity of the spirochetes is not known. He speculated on the likelihood of considerable leptospirosis in various parts of Jamaica on the basis of the neutral to alkaline pH of the soil, the surface and irrigation water, and the multitude of potential animal carriers.

In 1955 Bras (33), a pathologist, reported two cases of deeply jaundiced, hematuric patients, both comatose on admission, and both of whom died within several hours of admission to the University College Hospital. Leptospires were seen by Levaditi (silver) stain in the kidneys and liver of each patient.

In 1957 Grant and Bras (34) reported on the early efforts of the clinical bacteriology laboratory at the U.C. Hospital. 1) In 1956 the first leptospiral isolation from a patient in Jamaica was made. It was from a classical, severe case in a 17 year old gardener (35). This was identical to, or very closely associated with, L. kremastos, in the L. hebdomadis serogroup, previously not known in the western hemisphere. 2) In a serologic survey of 170 patients from various parts of the island (whose blood had been sent to the lab for V.D.R.L. testing) 3 positive sera were found. 3) The authors mentioned 3 positive cases of leptospirosis in dogs (? diagnostic technique). 4) From August 1953 through January 1957, 35 instances of human leptospirosis were shown by laboratory techniques among 200 suspected cases.

In 1964 Grant, Chen, and Urquhart (36) published a compilation of the experiences of the Leptospire Laboratory from January 1953 to March

1963. These included the serologic testing and cultural attempts on University College Hospital and referral patients; serologic surveys of humans around the island; serologic surveys of wild and domestic animals; and some animal cultural efforts. As of that time 14 isolations had been made from 170 blood specimens of "various origins." Six were identified as *L. kremastos*, 3 *L. icterohemorrhagiae*, 5 more were still being investigated.

Of about 500 patients' sera tested for antibodies to leptospires, 277 were found to be positive (174 males, 91 females, 12 sex not submitted with the specimen). The occupations of 50 of these were determined: 18 agricultural workers; 8 masons and carpenters (together); 5 students; 3 each, shopkeepers, domestics, housewives; 1 each, child, street cleaner, butcher, tailor, painter, cook, clerk, watchman, teacher, truck driver.

The authors reported the results of a several-year survey of 1950 sera collected all over the island. There is no mention of the criteria for selecting or method of collecting the sera. The sera were broken down by geographic location (i.e. parishes, which in Jamaica are roughly equivalent to small states or large counties on a U.S. standard). Over all 8 per cent of the sera (156 of 1950) were found to be positive, using both the rapid (macroscopic) slide agglutination technique and the agglutination-lysis (microscopic) test.* Of these 49 per cent were males, 34 per cent females, and 17 per cent sex unknown (not submitted

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*See materials and methods below.

with the specimen). When grouped by parish: Kingston-St. Andrew, 7.4 per cent positive (27 of 364); St. Catherine, 14.1 per cent positive (42 of 298).

Results of a serologic survey of 760 wild and domestic animals of unstated geographical distribution revealed 91 (12 per cent) positive to L. icterohemorrhagiae or L. kremastos antigens (Table 1).

Table 1

Animal	No. Tested	No. Pos.	Per Cent Pos.
cows	346	40	11.6
pigs	136	11	8.1
sheep	78	2	2.6
goats	72	9	12.5
horses, mules, donkeys	71	24	33.8
rats	30	3	10.0
mongooses	22	1	4.5
dogs	4	1	25.0
guinea pig	1	0	--

Recently Urquhart (37) has been conducting a preliminary survey of rats in the Kingston area. To date, five isolations of leptospires have been made from some 30 rats. Those thus far identified are either L. icterohemorrhagiae or L. kremastos.

In other parts of the British Caribbean a few studies on leptospirosis have been performed. In 1962 Downs and associates (38) reported finding both serologic and cultural evidence of the disease in Trinidad. They accidently came across the organisms while studying viruses. Leptospires accounted for 14 fevers of unknown origin out of 150 such cases. The

clinical pictures were generally mild. Antigens agglutinated by the sera included *L's*. *grippotyphosa*, *canicola*, *icterohemorrhagiae*, *medanensis*, *naam*, and *samarang*. Seven isolations were made from patients' blood. Among those identified two were *L. kremastos*, one *L. icterohemorrhagiae*, one (?) *kremastos*, one (?) *grippotyphosa*.

In other studies in the British Caribbean, Urquhart and Grant (39) in 1966 submitted "random" human sera to testing with a wide range of prepared antigens. The criteria for selection of sera, which were sent in from six islands plus British Honduras and British Guiana, were not stated. Approximately 50 specimens from each territory were examined. The per cents of positivity ranged from a high of 10 per cent for Antigua to a low of 2 per cent for Barbados. Results from Trinidad were 4.6 per cent positive as compared to Downs et.al's. value of 9.3 per cent positive among patients with fevers of unknown origin. Those serotypes whose antigens were most agglutinated were *L's. icterohemorrhagiae*, *canicola*, *bataviae*, and *australis*. Cross reactions involved *L. kremastos* and *L. sejroe*. With the small numbers of sera and the unknown criteria for selection of subjects and quantitative results of this study are of only partial reliability but the qualitative results are quite interesting.

Thus there is evidence that: 1) Leptospirosis is found in humans in the British West Indies, particularly in Jamaica; 2)persons of many occupations are affected; 3) various serotypes, especially *icterohemorrhagiae* and *kremastos*, are involved; 4) a large number of animals in Jamaica are subject to infection and may or may not serve as carriers;

5) the theoretically proper environmental conditions for the occurrence and transmission of leptospirosis -- correct water pH and numerous rodents (Guilbride (31)) -- are present on the island of Jamaica.

Since only preliminary epidemiologic studies have to date been made, it was decided to undertake a somewhat more systematic survey which might point the way toward a much larger study of the epidemiology of leptospirosis in Jamaica. Questions which must ultimately be investigated include: 1) What is the clinical picture of leptospirosis as seen in Jamaica? How serious a disease is it there? 2) What is the quantitative relationship of subclinical to clinical disease? 3) What is the incidence of the infection in the general population? What is the overall prevalence of positive serology? 4) What groups, occupational or geographic, are at highest risk? 5) Which are the important carrier animals? 6) Which leptospiral serotypes are active in man and/or animals?

Jamaica (39) an independent nation in the British Commonwealth, is the largest island of the British West Indies and lies about 80 miles south of the eastern end of Cuba. It is 144 miles long and 49 miles in maximum breadth and includes 4470 square miles. Spanning the island from east to west is a mountainous backbone which reaches as high as 7388 feet. The greater part of the island is composed of a platform of while limestone which is cut and riddled by small surface and underground rivers into strikingly beautiful hills, valleys, "cockpits," and caverns. The whole is covered by a lush tropical vegetation. On the south coast in the Kingston-Spanish Town areas is the Liguanea Plain -- a massive, relatively flat alluvial plain composed of limestone and acid to neutral

rock material.

The climate is subtropical with a temperature range from 70 to 80°F along the coast land, such as the Liguanea Plain. Generally there are two rainy seasons -- in May and in October -- but there is usually some rain every month.

The indigenous fauna on the island include various insects, scorpions, centipedes, some nonpoisonous snakes, many lizards, land crabs, and numerous birds and bats. In addition, fish, turtles, and the occasional corcodile are found. Domestic animals (horses, donkeys, mules, dogs, cats, swine, goats, sheep, fowl) have been imported. Rats (R. norvegicus and R. rattus) and various mice, common in country and town, were carried to the island by European ships. The mongoose, H. auropunctatus, which abounds in the rural areas, was introducted by the British from Southest Asia, reputedly to eliminate poisonous snakes and to control the rats and mice. The snakes were well suppressed, but mongooses sometimes pose a problem to chickens.

The population, estimated at 1,685,000 in 1964 (40), is predominantly of African origin. Roughly 95 per cent are racially pure or mixed Negro. The remainder consist of East Indians, Chinese, European (English, Scots), and persons of Middle Eastern origin.

Sugar has been among the most important industries since Spanish domination in the 16th century. It continued under the British with the use of African slaves. Into the 18th century the island was quite rich. After emancipation in the early 19th century cane production wavered and has only partly regained even with the importation of East

Indian and Chinese laborers in the later 19th century. Irrigation was introduced in the mid 19th century for agricultural purposes to the Liguanea Plain, an area which had been fertile but drought-stricken. This region, including the southern part of the Parish of St. Catherine, is still important in sugar as well as other crop production.

LEPTOSPIROSIS IN JAMAICA

A preliminary step in the survey was to attempt to determine the typical clinical picture of human leptospirosis in Jamaica. Because the disease is frequently seen at the University College Hospital (U.C.H.) in Kingston and because the Leptospira Laboratory has kept very good records since late 1962, the project was undertaken at the U.C.H. The additional information of seasonal incidence, relationship to rainfall, age and sex distribution could be determined from these records plus a few environmental data.

Laboratory records were gleaned by this author for all those inpatients at the U.C.H. and outpatients seen in the Casualty Ward who had either: 1) serologic evidence of leptospiral infection: a., macorscopic rapid slide agglutination positive (see materials and methods below) to _L. icterohemorrhagiae_ and/or _L. kremastos_ antigens; b., microscopic agglutination -- lysis (live antigens) test positive 1/100 or greater for these serotypes; c., or both; 2) isolation by blood culture of leptospires.

Information noted was (1) patient's name; (2) date of first serum or blood culture tested; (3) patient's age; (4) sex; (5) hospital number; (6) laboratory number; (7) reaction to each test -- the two serologic

tests and culture if attempted; and (8) apparent serotype predominating in the serologic tests (not of great reliability in the acute disease).

Using the hospital or casualty number, the patients' dockets (charts) were sought in the records room, professors' offices, etc. Those located were examined for the presence or absence of various classical signs and symptoms of leptospirosis. In all, 90 patients were located in the laboratory records who filled the selection criteria. This does not include several cases who, while having sufficiently high titers, did not have disease pictures at all compatible with current disease, as determined in the chart examination. A very few patients with positive serology from past infection may have been included if their dockets were not found to evaluate that.

Of these 90 patients the dockets on 45 were located. Those signs and symptoms mentioned in at least one place in the docket were considered positive. Negatives included those signs and symptoms recorded as such plus those not mentioned specifically anywhere in the docket. Not mentioned was interpreted as meaning not a significant part of the clinically observed picture. These data are presented in Table 2.

There was no way of determining how representative of the whole group these 45 studied cases were. It is conceivable, for example, that charts of those serious cases which came to post mortum were more consistantly filed in the records room.

But whatever the quantative limitations, the compilation should yield some reasonable picture of leptospirosis in the Kingston area when serious enough to receive medical attention. The Table's major limitation

is that those cases, if any, which are so atypical as to not suggest the proper diagnosis and therefore not come to the attention of the laboratory are not included. The clinicians at the U.C.H. are, however, quite familiar with the disease and are aware of its commoness and protean character, They routinely send to the laboratory sera from cases of apparent infectious hepatitis, typhoid fevers of unknown etiology, and occasionally encephalitis, as well as suspected leptospirosis patients.

Table 2

Compiled Signs and Symptoms of 45 Cases of Clinical Leptospirosis at the University College Hospital Compared with Two Other Series.

Signs and Symptoms	U.C.H. Series (45 cases)	Alston & Broom(U.K.) (600 cases)	Alexander(P.R.) (208 cases)
fever	89%	--	99%
anorexia	87%	--	--
vomiting	52%	--	69%
jaundice	73%	74%	49%
hepatosplenomegally	82%	--	69%
abdominal pain or tenderness	69%	--	--
proteinuria	59%	75%	25%*
lymphadenopathy	48%	--	24%
myalgia, arthralgia	66%	69%	97%
scleral injection	42%	72%	99%
headache	71%	87%	91%
meningeal signs	12%	40%	9%
pneumonic involvement	43%	--	24%
petechiae or rash	9%	55%	16%
cardiac failure	2+, 2±%	--	--
death	13%	--	6%

*Oliguria only, proteinuria not listed

The occupations of these 45 patients are presented in Table 3.

Table 3

Occupations of the 45 Patients with Diagnosed Leptospirosis

Occupation	Number	Remarks
Agriculture	14	
Laborer	8	Two of these work for the Water Commission.
Tradesman	5	All five admit to rats around place of work.
Domestic	4	
Child	4	
Housewife	2	
White Collar	2	
Unemployed	2	One admits to rats in his yard and to drinking unboiled river water.
Other	4	(watchman, truck driver, student, public health nurse.)

The 90 cases were then plotted by sex and age at which the disease occurred. The older age groups may have been enriched by a few cases whose antibody titers were from past infection. Figure 1 shows these findings.

In considering environmental or seasonal factors, the mean temperature change between summer and winter is only a few degrees. Much more important is the seasonal variation in rainfall. Figure 2 compares monthly rainfall with incidence of leptospirosis.

Figures on rainfall in the Kingston metropolitan area were acquired from the Government Meteorological Station at Palisadoes Airport, where daily statistics for many parts of Jamaica are kept. The actual figures used in this study were gathered at St. George's College Weather Station in Kingston. The rainfall statistics have been plotted by month from

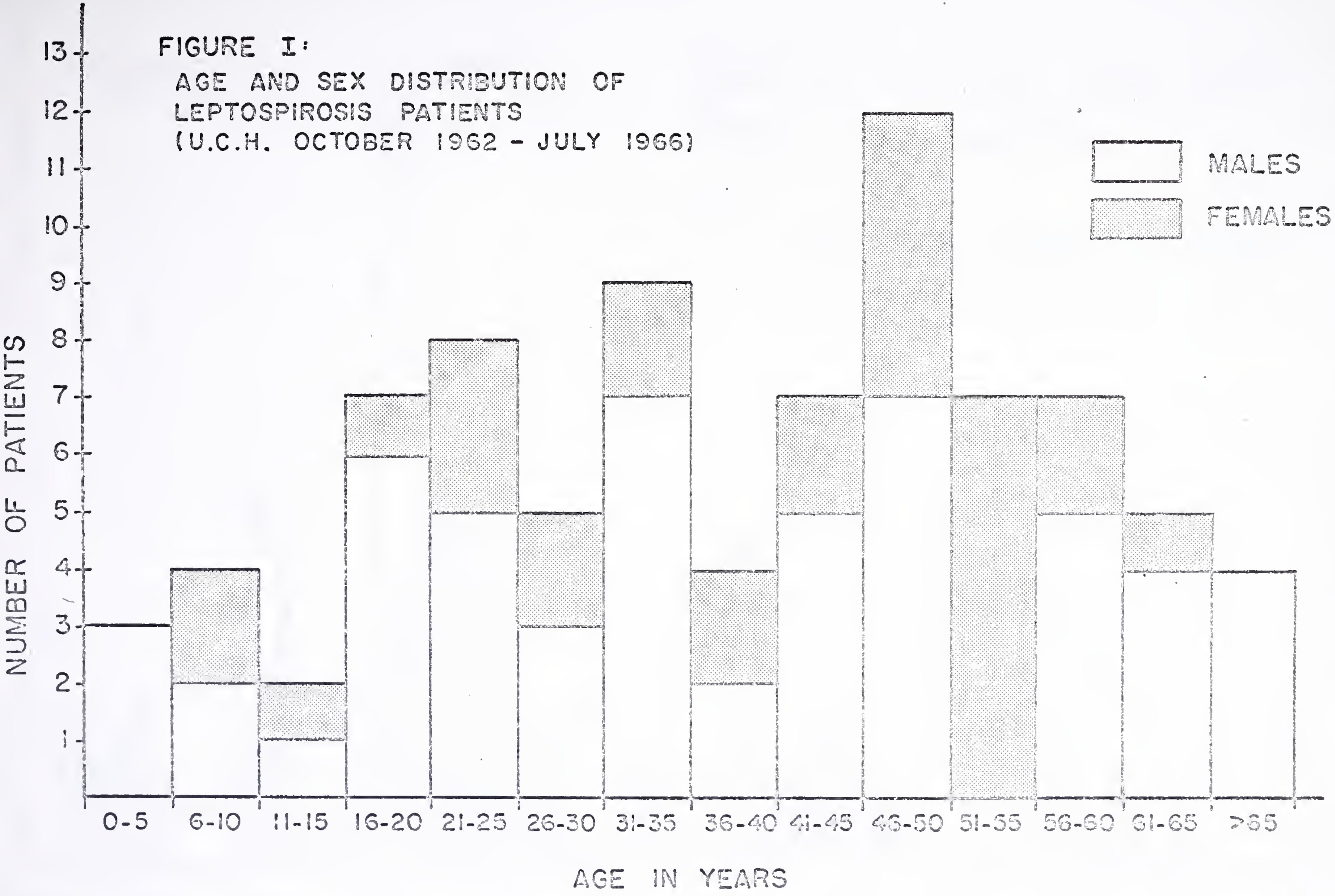
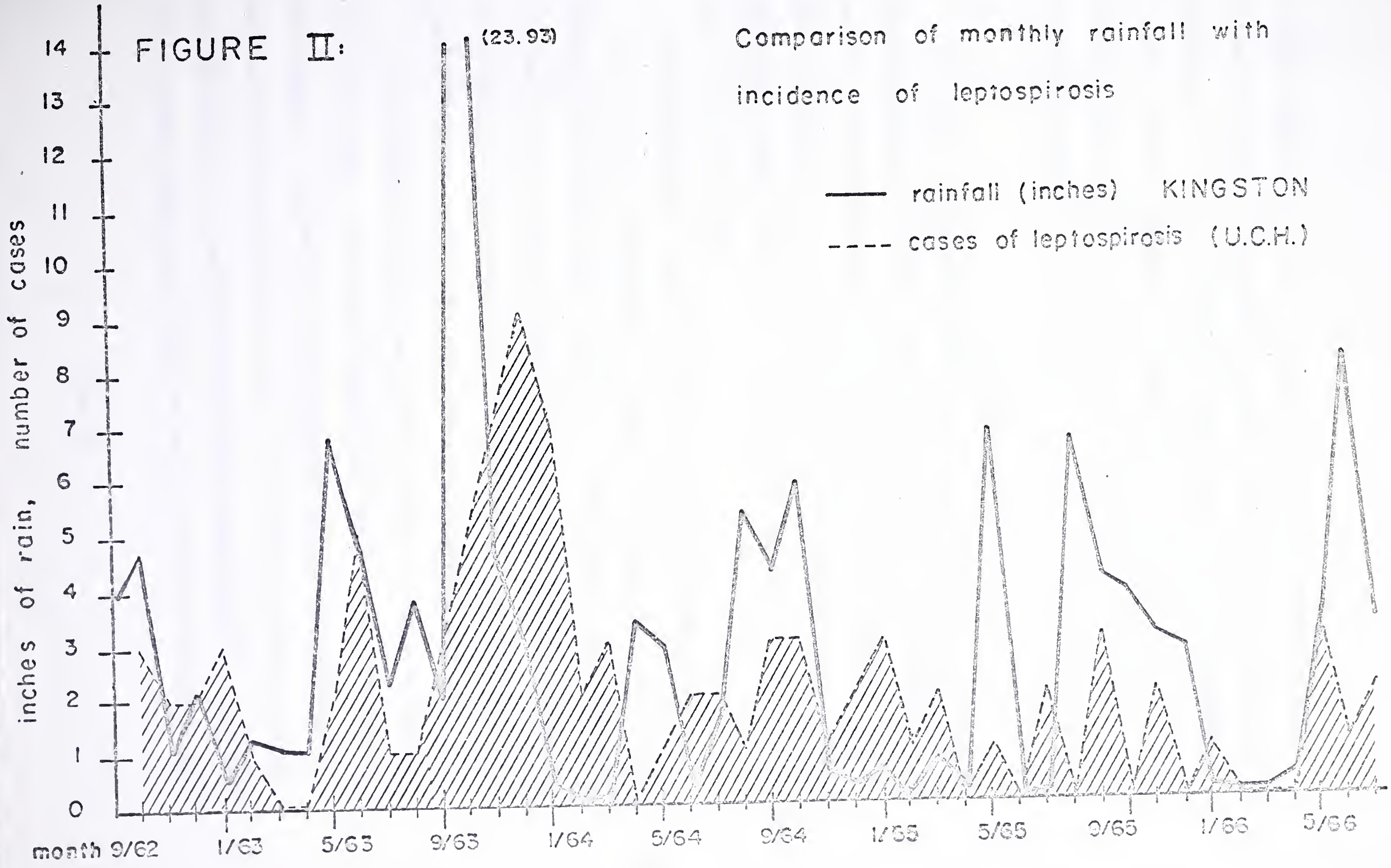


FIGURE I:
AGE AND SEX DISTRIBUTION OF
LEPTOSPIROSIS PATIENTS
(U.C.H. OCTOBER 1962 - JULY 1966)

FIGURE II: Comparison of monthly rainfall with incidence of leptospirosis



July 1962 through July 1966. The figures for monthly leptospirosis incidence are based on the date the earliest specimens were sent to the Leptospira Lab for cases eventfully demonstrated to have the disease. The usual time for appearance of antibodies is the seventh day (3), so that those first positive sera arriving at the laboratory indicate cases at least one week old. See Figure II.

DISCUSSION

The results of the investigation and compilation of clinical manifestations compare favorably with other such series. Two reported studies are tabulated along with the Jamaica findings (Table 2). The Alston and Broom series is based on 600 cases of leptospirosis in various hospitals in Great Britain (3). The Alexander series comes from 308 cases seen in Puerto Rico (24).

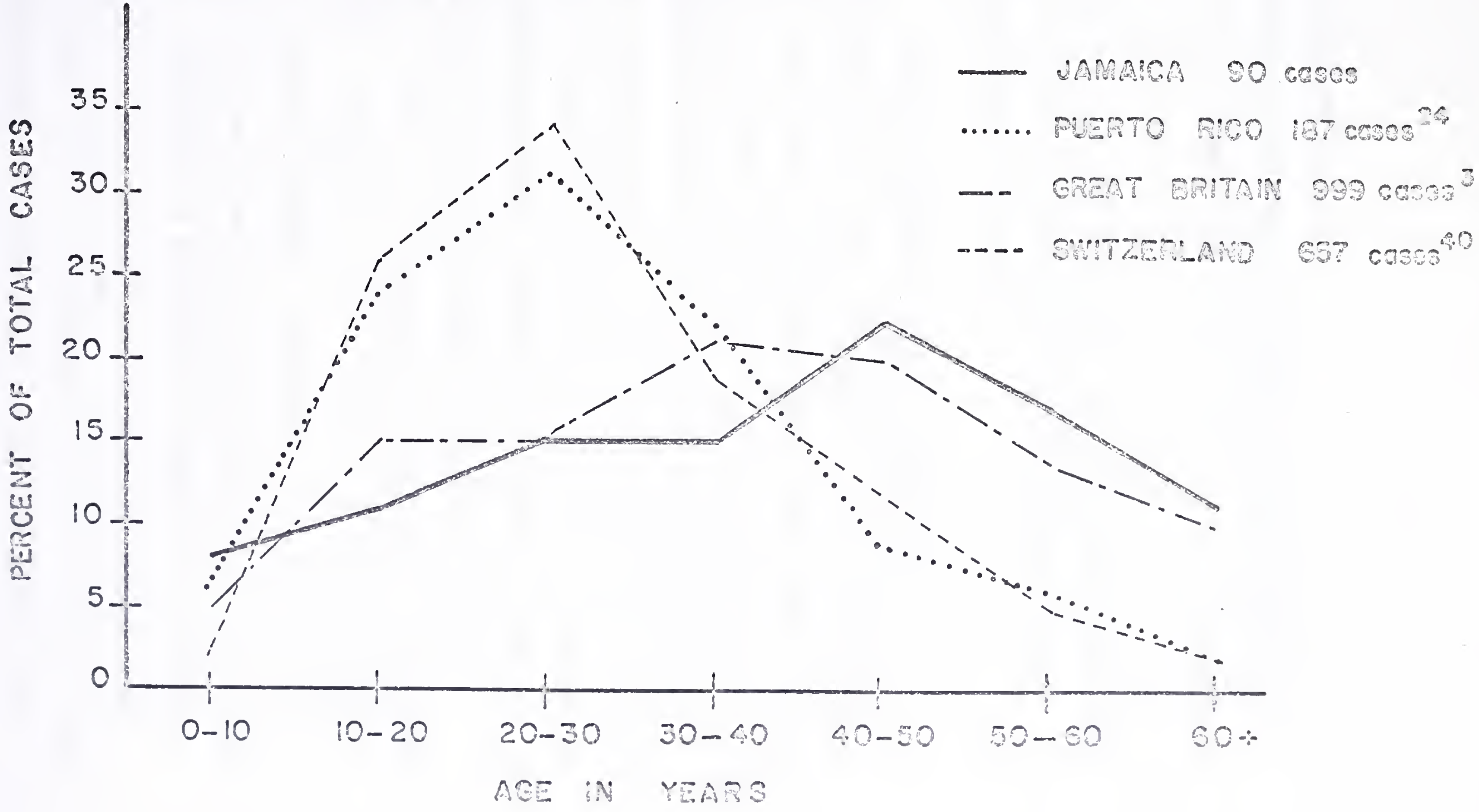
The occupational histories (Table 3) speak for themselves.

As to the age distribution, a series of 999 cases from Great Britain appears quite similar (1940 -- 1955) (3). The peaks of the British and Jamaican groups are at a later age than are those in the curves for a Swiss group of 657 cases (1944 -- 1951) (40), and the Puerto Rican group of 187 cases (24). The four curves are compared in Figure III.

In the Jamaican group a sex ratio of nearly 2:1 males to females (59:31) was found. Sex ratios usually tend toward males predominating over females. However, the ratios generally reflect the differential exposure rates of the sexes and are not considered a result of sexual predisposition. In some occupations such as pea harvesting in Northern Italy, women outnumber men among the workers and also in the relative

FIGURE III:

AGE DISTRIBUTION OF LEPTOSPIROSIS PATIENTS IN JAMAICA COMPARED WITH 3 PUBLISHED STUDIES



incidence of leptospirosis (3).

As to the apparent association in Jamaica between rainfall and the incidence of the disease, this trend was also seen in the Puerto Rican study (24).

Thus leptospirosis in Jamaica appears similar to the disease seen in other parts of the world in terms of clinical appearance, age distribution, and relation to rainfall.

SURVEY--ABBATOIRE, ESTATE, LABORATORY

During the first summer (1965) a preliminary survey was made for leptospirosis to help select a "population" for further study. Defining and working with a reasonably sized normal population would have technical complexities beyond the scope and time allotment of this investigation. Under conditions in most countries, a normal population might be expected to have quite a low prevalence of positive serology (3). In most places the disease has been customarily associated with certain occupations and activities, or at least found where rodent infestation is heavy.

The majority of the U.C.H. leptospirosis patients fell into several lower-class, outdoor occupations. Others admitted to having numerous rats around their places of business. Nonetheless, Grant et.al. (36) found 8 per cent of the sera from around Jamaica positive for past leptospiral infection. The parish of Kingston-St. Andrew had 7.4 per cent, St. Catherine 14.1 per cent. However, the criteria for selection of subjects are not clear. So it was decided in the present study to sample several classically higher risk groups and some of the animals they contact in order to find enough positives to analyze further.

The limited aims of the preliminary survey were to determine: 1) whether leptospirosis, or really serologic evidence of such, does exist in several occupational groups, which in other parts of the world have been found to be at risk; 2) whether animals in occupational contact with these groups show evidence of leptospiral infection; 3) an estimate of the prevalence of positive serology in the various human and animal groups; 4) which leptospiral serotypes are involved in infections of man and animals; 5) which human group would be most promising for further study, both because of high prevalence of positive serology and because of accessibility.

In addition to the search for anti-leptospire antibodies, V.D.R.L. testing was performed on all human subjects both as a service and as an inducement.

I. The first group were workers and frequent visitors at the Spanish Town Abbatoire. At this establishment, unlike at the quite modern facilities in Kingston, animal slaughtering is conducted in a rather traditional manner. Hundreds of cattle, swine, goats, and sheep are brought in each week from Jamaica's "country" parishes by "butchers." Many parts of the island were represented by the animals -- no one could tell the investigators exactly where the individual animals were from. At the abbatoire the beasts are slaughtered by "killers." Assistants then skin and evicerate the carcasses. "Porters" and "helpers" handle and carry the meat. "Tripe-strippers" and other specialists prepare the various byproducts. A Public Health Inspector examines each carcass and viscera for tuberculosis, lung worms, and liver flukes.

Cattle are slaughtered in an enclosed barn-like structure with a concrete floor containing a central trough. Blood, manure, urine, and refuse are swept down through this trough. The floor is washed periodically with buckets of water and brooms. Swine and smaller animals are butchered in a more outdoor structure adjacent to the cattle building. A majority of the killers, handlers, and others work barefooted and are in intimate contact with carcasses, blood, and refuse. In addition to employees, various onlookers, including barefotted school children, are constantly around and, at times, in contact with the animals. Several persons acknowledged that some rats and mice come around, at least at night.

The abbatoire was visited by the author and Mr. Brahim on three occasions. All those workers and onlookers who would consent were bled. The subjects were told they were being tested for "bad blood" (syphilis) and also for a fever carried by animals. Nearly all the males submitted to the test. One lady butcher consented, but none of the tripe-strippers, gut-cleaners, or other entrail handlers, most of whom were women, would participate. They did, however, send their children.

The subjects were informed one week later of the results of the V.D.R.L. test. Those requiring treatment were given appointments to the Spanish Town Health Center.

At the time of blood drawing the subjects were questioned as to name, home address, age, occupation, length of time employed, animals exposed to, and a brief clinical history including jaundice, meningitis, prolonged fever, dengue (for possible other use of the sera), hospitalization

and reason for it. Additional information, sex, race, study number, was recorded.

Animals slaughtered while the investigators were present were included in the study. (See method below.) Recorded were type of animal, sex, and approximate age. The original locality of these animals could not usually be obtained. In addition to the Spanish Town animals, a group of pigs and cattle from the Kingston Abbatoire were surveyed.

II. The second human group were associated with Jamaica's major agricultural industry, sugar cane cultivation and processing. The plantation selected for accessibility and theoretical likelihood of leptospirosis was the Caymanas Estates. Located in St. Catherine's Parish between Spanish Town and Kingston, Caymanas is an old estate of approximately 15,000 acres on the alluvial Liguanea Plain. Within its borders are numerous villages, several of which belong to the estate. An estimated 600 persons live on the estate itself. Several thousand others reside in the local villages and outlying areas. At crop period about 1800 persons are employed by the plantation, while only 1200 off season.

Great numbers of cane fields, growths of bananas and plantains, and some corn fields cover the estate. The Caymanas factory produces and refines sugar from the locally grown cane. In addition, the estate has a race track and golf course. Race horses are raised on the grounds.

The estate itself is a lowland plantation extending along the Spanish Town Road and stretching north and south from the seacoast to the Red Hills. Geometric fields of cane are divided by roads, canals, and rows of coconut palms. Requiring considerably more water than the local rain

provides, the cane fields are scored by thousands of parallel irrigation ditches. Major ditches course along the roads; lesser ditches branch off into the fields. Water runs in the canals and major ditches continuously and is let into the fields at appropriate intervals.

Irrigation water comes originally from the Rio Cobre in the limestone hills of St. Catherine. The river's pH is neutral to alkaline as is that of the canal water. The hills surrounding Caymanas are almost entirely limestone and marl (geologically recent calcareous rock not yet true limestone). Outcroppings of these rock formations stud the northern aspects of the estate.

Dogs, fowl, and occasionally pigs and goats can be seen around houses in the villages. There are several types of wild animals in the vicinity. People living in the villages claim that rats abound around the dwellings. Mice and rats are occasionally seen in the fields. However, the predominant denizens of the fields, by workers' estimates, are the mongooses. These readily visible animals live in the woods and fields and run rampant in irrigation ditches and among the rows of cane.

At the village of Caymanas, where the factory, church, community center, and store are located, stands the Caymanas Clinic building. A full-time nurse, Mrs. Carmelita Burton, attends daily. Once a week Dr. Ludlow Moody holds "doctor's clinic." Clinic services are extended to all employees and their families, and to all residents on the estate. Mrs. Burton estimates that about 3000 patients are registered.

Because the clinic is essentially the only gathering place for the scattered members of the estate community, it was decided most practical

to use a clinic population to survey for leptospirosis on the estates. All those patients of any age who would consent to the blood test were included. As a service to the subjects, the V.D.R.L. test was performed on all bloods.

This author and Mr. Brahim attended Dr. Moody's clinic day several times and drew blood on the patients as the doctor sent them in. As at the abbatoire the information on personal and occupational history, animal contact, and clinical history was collected. Results of the V.D.R.L. test were returned to Dr. Moody, who instituted therapy if indicated.

During the summer of 1965 a number of wild animals were trapped on the estate by the Field Virus group at the U.W.I. The blood of some of these animals (rats and mongooses) was made available for the leptospiral investigation.

III. Laboratory infections with leptospires have generally come from two sources: 1) handling or being bitten by infected animals during animal colony epizootics or by infected wild animals; 2) actual contact with leptospires in culture.

The third group surveyed for evidence of leptospiral infection included U.W.I. personnel who worked either with animals or with contaminated equipment.

Several persons in the Department of Microbiology work directly with the organisms. In handling contaminated equipment they routinely wear rubber gloves. Strong precautions are taken to disinfect apparatus and work surfaces.

Research personnel in Field Virology handle rats and other wild

animals in viral isolation procedures. In the past, leptospires have been cultured on occasion from such animals. Relatively few personal safety precautions are taken during bleeding and dissection of these animals. The animal carcasses are disposed of by porters who also employ no particular precautions.

A number of men hand-wash glassware in the Microbiology Department. Contaminated equipment is disinfected after use and autoclaved before washing. Furthermore, the washers wear rubber gloves.

For research purposes the University maintains animal colonies, including rats, mice, guinea pigs, rabbits, and sheep. A number of experienced men care for these colonies.

All those personnel found who in any way contact actual leptospires, wild or laboratory animals, or contaminated glassware and equipment, were included in the survey as the third group. The animals, other than those rats and mongooses trapped at Caymanas, were not examined. Subjects were treated the same as those in the other groups.

MATERIALS AND METHODS

Domestic animal bloods were collected at the time of slaughter from the spurting carotid arteries into clean, sterilized, rubber stoppered tubes labeled as to type of animal, sex, and age (juvenile or mature). Wild animals, rats and mongooses, were bled from the heart, employing antiseptic technique. Data on species, sex, approximate age, and locality where caught were recorded. Human subjects were bled where they worked or at the Clinic. The preliminary study information was collected.

All bloods were refrigerated overnight for good clot retraction.

The next day the tubes were "rimmed," the clots withdrawn, and the tubes spun at top speed on the clinical centrifuge for 15 minutes. Sera was pipetted off into aliquots in sterile screw-top bottles for serologic testing, V.D.R.L. testing, and reserve. Sera for leptospiral testing was stored at 4°C. Samples for V.D.R.L. testing, if not used the day separated, was stored frozen at -15°C.

Numerous techniques are available for examining sera for antibodies to leptospires. They vary in specificity, convenience, cost, hazardousness, and suitability to the intended purpose.

Tests include microscopic agglutination and lysis of living antigens, macroscopic agglutination of killed antigens, complement fixation using variously prepared antigens, hemagglutination and hemolytic tests employing erythrocyte sensitizing substance.

One of the oldest procedures is the microscopic agglutination-lysis test of Schüffner and Mochtar (42). Still widely used today, it is the standard for comparing other methods (41). The technique employs incubation of serum in various dilutions with live leptospiral antigens. The incubation mixtures are then examined under darkfield illumination and graded quantitatively for agglutination and lysis.

Both the advantage and the disadvantage of the test lie in the high degree of serogroup sensitivity. This requires a bank of antigens, including all the serogroups likely to be encountered. Even with the low grade cross-reactions that exist antibodies to an unknown leptospire can readily be missed.

Stocks of all the leptospires must be maintained with weekly dark-field examination and weekly subculture. Organisms for the test must be in the log phase for proper functioning. Each antigen requires calibration against standard antisera each time the test is performed. In the test each antigen is incubated, in duplicate, with various dilutions of each serum.

The major drawbacks of this technique are the laboriousness and expense of maintaining the antigens, the necessity of a permanent laboratory, the problems of standardizing antigen sensitivity, the tediousness of the test itself, and the personal hazards of handling live leptospires.

Several microscopic agglutination tests using killed antigens have been worked out. When the antigens have been properly prepared, standardized, and periodically checked for sensitivity, the test systems have proved essentially equivalent to the agglutination-lysis technique (41). However, the individual antigens required must generally be prepared in the laboratory using them. They do not keep well and have proved somewhat unpredictable in preparation and storage (37).

In 1958 Galton et.al. (43) developed a much simplified antigen system. Using formalinized leptospira suspensions they perfected a rapid macroscopic slide agglutination test analagous to previously used systems for Brucella, some Salmonella and Pasteurella. The investigators eventually developed antigens for 12 serotypes found in the U.S. With these they could reproduce the sensitivity and, more or less, the group specificity of the live antigens.

The antigens are now available commercially (Difco Laboratories) and

are widely employed by clinical and veterinary laboratories for the serodiagnosis and survey of leptospirosis. When properly handled and refrigerated, the antigens are stable for at least 18 months (44). They are relatively inexpensive and fairly simple to use. In addition to the original 12 serotypes, L. kremastos and L. biflexa are now available. For rapid screening the original 12 are grouped into four pools of three each.

To perform the test (44): 0.01 ml. of undiluted serum is pipetted onto a ruled glass or clear plastic sheet, one drop (about 0.055 ml.) of antigen is added from the supplied dropper, the serum and antigen are mixed briefly with a toothpick. The plate is rotated by hand six to eight times; it is then placed on a mechanical rotator for 4 minutes at 125 rpm. The reaction mixture is observed over a light source for the presence of agglutination. With a positive or doubtful reaction to pooled antigen the test is repeated with the various individual antigens. Reactions to specific antigens are graded 0 to 4+ on the precentage of antigen agglutinated. For titration (performed only with specific antigens) sera are appropriately diluted and the tests repeated.

While the various agglutination tests are reported as highly serogroup specific, host responses apparently are not necessarily so. In addition to cross-reacting antibodies which can be adsorbed out in vitro, another phenomenon is occasionally observed. For example, in culturally proved L. icterohemorrhagiae infection, antibody to L. canicola may rarely be in higher titer than antibody to the infecting organism (41). The same thing occurred in a Jamaican study (36) in a case of culturally

demonstrated L. kremastos infection. In the first week of illness the serum agglutination-lysis titer against L. icterohemorrhagiae was 1/300, but negative against L. kremastos. Two weeks later the titer against L. ictero was 1/3000, and against L. kremastos 1/1000. This phenomenon has often been observed early in the disease course and is not considered simply due to double infection. It is the so-called "paradoxical reaction" of Führer (45). Usually later in the disease or convalescent period the homologous titer gains ascendency. Moreover, fresh convalescent sera, in general, will agglutinate a number of heterologous antigens though usually less strongly than the homologous antigen. Thus the serodiagnosis of leptospiral infection gives good but only presumptive evidence as to the serotype involved. Isolation and identification of the offending organism remains the only sure method.

In a region with leptospires the normal person never yields a positive agglutination with either the microscopic and macroscopic techniques (3,43). Starting from about the sixth to the twelfth day of illness, the antibodies become detectable, typically in low titer. By the third or fourth week the titers may reach 1/10,000 to 1/30,000 by the agglutination-lysis test. In the absence of a history suggestive of the disease 1/300 is considered diagnostic of current infection. Ideally, sequential sera should be examined. Less than 1/300 in the absence of current symptoms is thought on epidemiological grounds to represent past infection.

In man, but not certain animals, detectable antibodies last a very long time. Alston and Broom (3) examined sera as late as 15 years after recovery, at which time all were still positive. They report other studies

showing positivity up to 16, 22, and 28 years. The macroscopic rapid slide agglutination test has not been thoroughly evaluated for its sensitivity to low titers in later years.

For the 1965 preliminary survey the collected sera were screened with the Difco microscopic agglutination test (of Galton et.al.). The pooled antigens were used:

Pool 1 -- L's. ballum, canicola, icterohemorrhagiae
Pool 2 -- L's. bataviae, grippotyphosa, pyrogenes
Pool 3 -- L's. autumnalis, pomona, sejroe
Pool 4 -- L's. australis, hyos, mini (georgia)

The decision to use these simple antigen systems was based on the limited personnel and time available and because of the expense, technical complexity, and limited antigen range of the microscopic agglutination-lysis test.

It was originally thought that the 12 serotypes in the pools would provide adequate screening for antibodies to most leptospires to be encountered. With undiluted serum there is cross-reaction with a number of antigens by the specific antisera tested (44). As to antibodies to L. kremastos, it was expected that these would be picked up by L. sejroe antigen (which is also in the L. hebdomadis serogroup and shares numerous common antigens). More distant cross-reactions, such as with L. icterohemorrhagiae, might also be expected to pick up antibodies to L. kremastos. Cross-reactions with various pools are seen at the U.W.I. in L. kremastos infections. At a later time the sera were screened also with kremastos

antigen, this serotype being so important in Jamaica. No additional positives were located with this antigen.

The pooled antigens used were brought to Jamaica especially for the survey. This was all that was planned for testing the first summer, merely to find a group with seropositivity for leptospiral infection, regardless of the serogroups involved. However, some specific antigens were available at the Leptospira Lab, having been left over from an earlier survey. _L's icterohemorrhagiae_ and _kremastos_ antigens were employed in routine laboratory testing as well. Positive reactors to pooled antigens were tested for activity to those specific antigens which were available. Many were still fresh but some were past the time limit suggested by the manufacturer. Positive and negative control sera was tested with each antigen.

RESULTS AND DISCUSSION

Group A -- Abbatoire Animals and Workers

Table 4

Results of Serologic Testing of Domestic Animals from Abbatoires

Abbatoire	Animal	No.Tested	No.Pos.	Per Cent Pos.	Serotypes Agglutinated
Sp. Town	goats	12	2	17	ictero, canicola, australis
	pigs	10	2	20	pomona, australis, autumnalis
	cows	10	1	10	canicola
Kingston	goats	2	0	--	
	pigs	33	2	6	canicola
	cows	3	0	--	

The animal findings at the Spanish Town Abbatoire, while based on very small numbers, are within an order of magnitude of the findings of Grant et.al. (36) on large numbers of Jamaican domestic animals. The present positive findings do indicate that the animals being processed at the Spanish Town Abbatoire have had contact with leptospires. It might be expected that at times animals with current infections are slaughtered, exposing the employees and the watchers to infection.

Table 5

Results of Serologic Testing of Abbatoire Personnel, Listed by Occupation

Occupation	No. Tested	No. Pos.	Per Cent Pos.
animal handlers	30	1	3.3
onlookers	8	0	--
unassociated	2	0	--
Total	40	1	2.5

Findings on the people exposed are not striking. One definite serologic positive was demonstrated. This man denied, even on requestioning, history of jaundice, meningitis, prolonged fever, severe muscle pain or hospitalization.

Group B -- Caymanas Estates Personnel and Trapped Animals

Table 6

Results of Serologic Testing of Sugar Estate Personnel, Listed by Occupation

Occupation	No. Tested	No. Pos.	Per Cent Pos.	No. Doubtful
field work	28	1	4	2
non-field (factory, etc.)	9	0	-	1
housewives	6	0	-	0
children	2	0	-	0
Total	45	1	2	3

Among the humans, there was one individual strongly seropositive. He was a nine-year veteran of the sugar fields. However, he reported a negative history for the outstanding clinical features of leptospirosis.

Table 7

Results of Serologic Testing of Wild Animals from Caymanas Estates

Wild Animal	No. Tested	No. Pos.	Per Cent Pos.	Serotypes Agglutinated
mongoose	7	3	43	canicola (2) ictero (1)
Rattus norvegicus	1	0	--	
(rats from another parish)	(2)	(0)	(-)	

The small number of animals acquired for study during the summer make quantitative results of questionable significance. But the fact that so many mongooses in this small number were seropositive is perhaps meaningful. They have been found to be carrier animals in other studies (20 per cent carriers and 51 per cent seropositive in Puerto Rico (24); 14 per cent carriers, 29 per cent seropositive in Hawaii (27)). Grant et.al. (36) previously found one of 22 Jamaican mongooses seropositive. Considering the commonness of these animals in the irrigated fields of the plantation, the possibility arises that mongooses may be important carriers here.

Yet only one out of the three positives agglutinated L. icterohemorrhagiae antigen. The agglutination of L. canicola by two of the sera is interesting, but human cases of that infection have not so far been demonstrated in Jamaica. This infection is, however, suggested in domestic animals by serologic findings.

Group C -- Laboratory Personnel

Of the 21 persons having contact with animals or contaminated

equipment. None were serologically reactive to leptospiral antigens.

Some of the wild animals handled by about half of this group (with negligible precautions) have been demonstrated to be seropositive and to harbor leptospires in this as well as previous studies (36,37). Nothing is known about possible leptospirosis outbreaks among the laboratory animals.

1966 SURVEY AT CAYMANAS ESTATES

Because of the interesting finding of seropositive mongooses and at least one seropositive cane worker at the estates, it was decided to concentrate the second summer's effort on this place. The possibility of the mongoose as an important carrier in Jamaica had not been investigated before. The economic importance of the sugar industry on the island and the large numbers of workers so employed was another consideration. Caymanas Estates would serve as a model, though how representative of other plantations was not determined.

A clinic population, an expansion of the preliminary survey group, was chosen on the basis of convenience and accessibility to the otherwise scattered personnel of the estate. It was hoped that enough subjects could be tested to include a large number of seropositives. With such a collection of positives, considerable information could hopefully be derived: (1) prevalence of antibody to leptospirosis in the estate (clinic) population, (2) age and sex destribution of seropositivity in this group, (3) relationship of a compatible history with serologic evidence of leptospiral infection (this would suggest a ratio of subclinical to clinical disease), (4) whether field contact places one more at risk for

infection, (5) whether dwelling near surface water increases one's risk to infection, (6) which villages on the estate, if any, are more at risk, and (7) which one or more leptospiral serotypes are involved on the plantation.

A simultaneous survey of wild animals was planned to include serologic testing and attempted culture of leptospires. This would suggest: (1) which wild animals have had more than only casual past contact with the organisms, (2) which animals, if any, remain carriers, and (3) by culture which leptospiral serotypes are involved.

MATERIALS AND METHODS

Patients coming to the Caymanas Clinic for any reason and who could be induced to participate by the clinic physician were included. I attended the clinic every week and bled and questioned each subject. The procedure was similar to that the previous summer. V.D.R.L. results were returned each following week to the patients and treatment was instituted by Dr. Moody when indicated.

A more extensive, printed questionaire (see appendix) was used this summer rather than the sparse screening questions employed the previous year. Information included personal data; reason for coming to clinic; home situation -- location, length of time there, nearness of house to various types of surface water, animals at the house; occupational situation -- type of work, length of time employed, occupational animal contact; clinical history -- jaundice, "hepatitis," "meningitis," prolonged fever, "Weil's Disease," "Dengue fever," hospitalization and reason for it. (Most subjects the previous summer had complained of

muscle ache, so this question was eliminated.) Any positive or suspicious point in the clinical history was further explored.

For catching animals a string of 11 live animal traps of the spring-closed box type and the door-dropping "Hav-a-hart" type were collected at the U.W.I. Various baits were used, including smoked red herring, salted mackerel, and salt codfish. The baits and traps chosen were considered appropriate for capturing rats and mongooses alive. Small field mice, which live in unknown quantity on the plantation, are not usually caught in these traps. The experimental protocol provided that traps be set in various parts of the estate so that a broad sample of the local animals might be gathered. Traps were to be visited by this worker three times a week. Live animals were to be brought back to the laboratory and the traps rebaited when necessary. In general, the traps were to be set out near streams and irrigation ditches, in cane fields, grazing fields, and nearby woods, as well as around human dwellings.

In the laboratory, species, sex, appropriate age, and locality caught were recorded. Then the animals were held firmly with tongs but not anesthetized, the ventral fur was thoroughly cleansed with copious 70 per cent alcohol, and as much blood as possible was withdrawn by cardiac puncture into a sterile syringe. The blood was placed into sterile tubes for preparation for serologic testing. The exsanguinated animals were then cleansed again with alcohol. With sterile dissecting instruments the kidneys were removed into a sterile screw top jar.

Human and animal sera were prepared and tested against the bank of pooled antigens as in the previous survey. At a later date the human

sera was screened also against L. kremastos antigen to be sure that no low titer positives had been missed by the pooled antigens. All positive reactors were tested against a number of specific antigens, including L. kremastos at the time.

Animal kidneys were inoculated into Fletcher's semi-solid medium for isolation of leptospires (see appendix for technique and medium components). Specimens of both kidneys were homogenized in cold, sterile, buffered saline. Inoculations in a range of dilutions were made into screw top tubes containing the medium. The tubes were incubated in the dark at room temperature (25-27°). Weekly examination under dark field illumination for leptospires was conducted for 5 weeks. Isolations were subcultured into the same medium. These were repeatedly further subcultured when at approximately peak growth. At the end of 5 weeks any negative cultures were discarded.

RESULTS

One hundred sixty-eight subjects were tested at the Caymanas Clinic between the periods of July 5 and August 23, 1966. They included all those patients or persons accompanying patients who would submit to the "blood test." The group ranged from age 5 to 78. They included estate employees, their relatives, and persons living on the estate, some of whom worked off the plantation. (The outside occupations ranged from hotel table waiting to inspecting at a brassiere factory.)

Summarized in Tables 8 and 9 and Figure III below is the group's breakdown by age, sex, home location, occupation, and chief complaint at clinic on the day tested. (In addition, those 36 patients from 1965

who did not reappear in 1966 are included in the tables. The 1965 group had not been asked for certain data, namely house proximity to water, animals at the house, and chief complaint at clinic.) Results of the serologic testing are compared with the data in the tables.

Table 8

Chief Complaint at Clinic of Caymanas Estates Personnel Studied Serologically

Chief Complaint	#M	#F	M & F	% of Total
Trauma, Orthopedic, Infection	28	14	42	25
for blood test	24	11	35	21
Pains, Headache	13	15	28	17
Dermatologic	6	6	12	7
non-patient (accompanied a patient, works there, etc.)	2	8	10	6
chronic disease care	4	4	8	5
Venereal Disease	7	0	7	4
"Cold"	5	0	5	3
"check-up"	4	1	5	3
Fever	4	1	5	3
Pregnancy	0	5	5	3
Malaise	1	3	4	2
other	2	0	2	1
Total	100	68	168	100

Table 9

Results of Serologic Testing of Caymanas Estates Personnel Compared with Home Location

Village	#1966		#1965		Total	# Pos.	% Pos.
	M	F	M	F			
Braeton	1	0	0	0	1	--	--
Caymanas	21	25	6	3	55	1	2
Caymanas Bay	3	7	2	0	12	--	--
Central Village	13	7	3	1	24	--	--
Christian Pen	3	5	1	1	10	1	10
Cumberland Pen	2	1	2	0	5	2	40
Ferry	1	1	1	0	3	--	--

Table 9 continued

Village	#1966		#1965				
	M	F	M	F	Total	# Pos.	% Pos.
Glade	1	3	0	0	4	--	--
Gregory Park	20	14	2	0	36	--	--
Newlands	4	0	1	0	5	--	--
Sligoville	1	0	1	0	2	--	--
Spanish Town	13	0	4	0	17	--	--
Thompson Pen	2	0	1	0	3	--	--
Waterloo District	2	0	1	1	4	--	--
Whitemarl	2	3	1	0	6	1	17
Kingston	10	2	3	0	15	1	7
Other	1	0	0	1	2	--	--
Total	100	68	29	7	204	6	3

Table 10

Results of Serologic Testing of Caymanas Estates Personnel Listed by Occupation and Sex

Occupation	#1966		#1965				
	M	F	M	F	Total	# Pos.	% Pos.
irrigators	4	1	2	0	7	1	14
field workers, total	58	15	24	1	98	4	4
factory, clerical, off plantation, etc.	34	5	5	1	45	2	4
housewives	0	24	0	2	26	--	--
children - school	6	15	0	2	23	--	--
domestics	0	8	0	1	9	--	--
non field, total	42	53	5	6	106	2	2
Total	100	68	29	7	204	6	3

In Table 11 are summarized the more specific details from the serologic testing and the questionaires for those six subjects whose sera agglutinated the pooled antigens. (The case from 1965, J.R., was not questioned as extensively on certain matters as those from 1966.

Thus all the subjects lived on or near the plantation (including the one from Kingston, who up until 9 months before testing resided at Caymanas Bay). All lived near either a river, canal or swamp. All questioned (not J. R. from 1965) had a dog at the house. Three had other domestic animals. Four worked in the fields; one of these worked in the canal water as an irrigator. One of the two non field workers grew up on the estate and one year ago began an outdoor job digging out trash at the sugar factory. One had a totally indoor occupation.

One had a strongly suggestive clinical history for leptospirosis (D. D.). One had a compatible history (E. E.). Three had negative histories for typical leptospirosis symptoms (G. M.$_1$, J. R., G. M.$_2$). The last (M. B.), a very poor historian, mentioned "typhoid." (Typhoid does occur in St. Catherine's Parish.) This history, poor in quality, is only questionably indicative at best.

Four were seropositive to Pool I (*L's. icterohemorrhagiae*, *canicola*, *ballum*) and specific *icterohemorrhagiae* antigens. One of these four gave a 1+ cross-reaction with *canicola* antigen. Two were seropositive to Pool IV (*L's. australis*, *hyos*, *mini - georgia*) and specifically *L. australis*. All six were negative to *L. kremastos*.

Table 11

Details on Seropositive Subjects with Regard to Serologic Testing and Clinical History

Pt.	Age	Sex	Village	Near Water	Animals at Home	Occupation	Serotype Agglutinated	Clinical History	Remarks
D.D.	17	M	Caymanas, 12 years	Canal	Dog	Factory, digs out trans, 1 yr. (grew up on estate)	Pool I 1+ *ictero* 2+	Hospitalized for high fever 1 yr. ago. Had myalgia, headache. No jaundice, meningitis. No diagnosis made.	
G.M.$_1$	26	M	Kingston 13. Lived at Caymanas Bay until 9 mo. ago.	River	Dog, goat cow	Operator in factory, never in field. 8 yrs.	Pool IV 2+ *australis* 3+	Neg. history. Hospitalized for ankle trauma as a child.	
J.R.	28	M	White Marl (? years)	(village is on river)	(?)	Field work 9 yrs. Now ass't tractor oper.	Pool I 2+ *ictero* 3+	Neg. History. (Even with re-questioning.)	tested 1965
E.E.	34	M	Cumberland Pen 10 yrs.	River	Dog, goat, cow, horse	Cane cultivator 10 yrs.	Pool I 1+ *ictero* 1+	"Malaria" 5 yrs. ago without jaundice, hospitalization. (No malaria in St. Catherine's in past 7 yrs.)	
G.M.$_2$	53	M	Cumberland Pen 15 mo. Newlands before that (near the coast.)	Canal	Dog	Irrigator 24 yrs.	Pool IV 1+ *australis* 3+	Neg. history. Hosp. E.R. for accident.	
M.B.	65 (?)	F	Christian Pen 10 yrs.	Swamp	Dog, pig	Field work 15 yrs. (retired (?))	Pool I 1+ *ictero* 2+ *canicola* 1+	Very poor historian (chronic brain syndrome) She mentioned "typhoid."	

ANIMAL SURVEY

A number of unexpected problems complicated the animal collection program. Stolen and lost traps, animals breaking out of the older traps, animals dying in the hot sun, self-closing of traps during rainy periods, and floods on the plantation limited the success of collection.

Over the 8 week period 8 mongooses and one rat were caught alive. None were seropositive, however, leptospires were isolated from one mongoose and the rat. The isolates have been subcultured repeatedly in Fletcher's medium. Identification of serotypes is still pending.

Table 12

Species	No.	No. Seropos.	No. Cult. Pos.	% Cult. Pos.
Mongoose (*H. auropunctatus*)	8	0	1	12
Rat (*R. norvegicus*)	1	0	1	100

DISCUSSION

I. Prevalence of antibodies to leptospiral antigens.

The results -- 3 per cent overall in this clinic population of roughly 200 persons (1965 plus 1966) -- are markedly different from the results of Grant et.al., in their all-Jamaica serologic survey (36). They found in about 2,000 specimens from various parts of the island 8 per cent positive (156 of 1,951). In the parish of St. Catherine 14.1 per cent were positive (42 of 298). However, no information was given on the selection of subjects for the survey. They were not stated to include a group of known or clinically suspected cases of leptospirosis,

or persons at high risk. If anything, the Caymanas group might be expected to have a higher rate of positivity than the general population.

It might be suggested that the antigen systems used by Grant et.al. (agglutination-lysis and rapid slide agglutination tests with antigens of L. icterohemorrhagiae and L. kremastos) were more sensitive than the pooled, rapid slide antigens used for screening in the present survey. However, in their paper the authors compared results with their two antigen systems on 100 clinical cases (how selected?). All 100 were positive using the rapid slide agglutination test; 60 were positive, 15±, and 25 negative by agglutination-lysis. This suggests either that the macroscopic agglutination test is more sensitive or more apt to give false positives than the agglutination-lysis test.

There are no figures to compare the relative sensitivity of the pooled macroscopic antigens with the individual ones. But it is not uncommon to find a 1+ or 2+ reaction with the pooled antigen and a stronger reaction with the specific antigen.

Thus while the specific antigen systems employed by Grant et.al. might be slightly more sensitive to the two serotypes sought, the pooled antigens in the present study are considerably broader in spectrum.

In the present human study, two strong positive reactors to L. australis antigen were found. Both were negative to specific L. icterohemorrhagiae and L. kremastos antigens. L. australis has not so far been isolated from either humans or animals in Jamaica, and is not routinely tested for serologically. Antibodies to this serotype were detected among domestic animals in this study (one goat and one pig).

The present overall results are more consistant with the 1957 results of Grant and Bras (34). These authors analyzed 170 sera sent in to the U.W.I. from various parts of the island for V.D.R.L. testing. Using the agglutination-lysis and complement fixation tests with L. icterohemorrhagiae and L. canicola, they found three positive sera -- 1.8 per cent. Since these sera represent a reasonably random population in terms of leptospirosis, the per cent of positivity compares with a 3 per cent prevalence on a sugar plantation where there are apparent carrier animals.

Among those butchers and other actual animal handlers at the Spanish Town Abbatoire, one of 30 (3 per cent) were positive.

II. Relationship of a suggestive clinical history for leptospirosis with seropositivity.

Any retrospective study of this sort has its limitations. Yet this clinic group presented additional problems, including lack of experience with extensive medical history taking, occasional apathy, a language barrier, and the apparent tendency for some Jamaicans to answer what they think the doctor wants to hear.

The small number of seropositive cases, six at Caymanas, make extrapolation of results unreliable. Among this group three gave negative histories, two gave suggestive histories, and one gave a confused history which could not be evaluated. The seropositive subject at the Spanish Town Abbatoire in 1965 had denied any history suggestive of clinical leptospirosis even on requestioning. None of the seropositives admitted to jaundice or meningitis while some of the seronegatives did.

These limited findings suggest that perhaps over half of the human

leptospiral infections on the estate are subclinical. None of those positives found appear to have had the serious clinical course often seen at the University College Hospital.

III. Whether field workers are more at risk for leptospirosis than non field workers.

Of the field workers 4 per cent were serologically positive (four of 98). Overall the non field workers (including school children) were 2 per cent positive (two of 106). Among the 45 factory and clerical workers 4 per cent (two) were positive.

However, further inspection of the positive "factory" workers reveals that one of them was a 17 year old boy who previously had worked at the Caymanas factory and had lived for years on the estates.

The data are insufficient to incriminate any particular type of work among this group as exposing individuals to higher risk of leptospiral infection. However, there may be a suggestion that infection is more frequent among field and other outdoor workers.

IV. Whether living in close proximity to water has a relationship to evidence of infection.

It is interesting that all five seropositives in 1966 lived "close" to some form of surface water or sewer (102 of 168 persons in 1966 admitted to living near water). However, with poorly defined limits for "closeness" no conclusions are justified.

It would be important in evaluating the significance of proximity to water to know how much actual physical or dietary contact this proximity implies for the persons or their domestic animals.

V. Which villages, if any, are more at risk toward leptospirosis.

While some of the villages are poorly represented in the survey, it is interesting to note that Cumberland Pen, from where there were only five subjects, had two seropositives. These were both field workers and as such had occupational contact with many parts of the plantation. Cumberland Pen is a small settlement on the flat lowlands in the south part of the estates near the coast. While it has some small streams, canals, and agricultural fields, the ground water is described as being too saline for leptospirosis (31). Yet the region tends towards local flooding during heavy rains, much as in the surrounding lowlands. The apparent high prevalence of seropositivity in this village is based on many too few data to be other than an interesting observation. However, a survey of persons, animals, and perhaps water in this lowland region might be rewarding.

VI. Age and sex distribution of the seropositive cases.

The only point with regard to age distribution of the seropositive cases is that a large age range shows evidence of infection by leptospires (Table 2). Of course a large age range was seen clinically at the U.C.H.

The sex ratio of the seropositive cases was five males to one female. The sex ratio of all the subjects was 1.7:1. However, among the field workers only, the seropositives were 3:1 males to females, whereas the sex ratio of field workers in general was 5:1 (82:16). Thus the field workers reflect the general observation that the sex ratio usually found results from differential occupational contact with contaminated water

and animals.

VII. Leptospiral serotypes involved in human infection.

Serologic reactors were found to only two antigen pools and correspondingly to two specific antigens. Reactors to L. icterohemorrhagiae had often been noted before. The reactors to L. australis antigen are a new observation in Jamaica. Previous human surveys and clinical testing there employ or have employed antigens of L's. icterohemorrhagiae, canicola, and kremastos. These two positives to L. australis were negative to specific icterohemorrhagiae and kremastos antigens and Pool 1, which contains L. canicola, and as such would presumably have been missed in previous surveys.

No positive reactors were found to L. kremastos antigen. (Although screening of those sera negative to the pooled antigens was not performed until about 9 months after they were collected.

The qualitative system and small number of antigens available for use in testing necessarily limit the reliability of the evidence for L. australis infection in man in the survey. More extensive, quantitative serologic testing and isolation of the organism must be performed to completely establish that this serotype is present in Jamaica.

VIII. Serologic evidence of leptospiral infection in wild animals on the estates.

In the 1965 survey three mongooses of seven caught at Caymanas were found to have antibody to leptospiral antigens. (One rat was negative.) These animals have been demonstrated to be numerically significant carriers of leptospires in only two places, Puerto Rico and Hawaii. And even their

relevance to human infection has not been evaluated. None of the eight mongooses and one rat caught in 1966 were seropositive. This is somewhat surprising in light of the previous summer's experience.

However, as discussed above, certain animals, notably the rat, can have antibodies following infection fall below detectable levels while retaining leptospires in their kidneys -- the "external" parasitism of Babudieri. That this phenomenon also exists in mongooses is suggested by the findings in Hawaii of Minette (27). Of 126 mongooses (*H. auro punctatus*), 18 yielded leptospires in kidney culture (14.3 per cent). Of these 18 cultural positives, only nine (50 per cent) were positive serologically. Thus the absence of detectable antibody does not rule out either past infection or current harboring of leptospires in these animals.

IX. Carrier animals.

Insufficient animals were captured to indicate reliably the prevalence of carriers of leptospires on the estates. Yet the organisms were isolated from both a rat and a mongoose. The cultured leptospires have not yet been identified as to either serotype or pathogenicity. It is significant qualitatively, however, that both types of animal do carry leptospires at Caymanas.

No investigation of domestic animals on the estates was made either for antibodies to leptospires or for isolation of the organisms.

X. Leptospiral serotypes infecting animals.

Of the three positive mongooses from 1965, two carried antibodies to *L. canicola* antigens and one, antibodies to L. icterohemorrhagiae. As mentioned above, serologic tests are not necessarily reliable for serotype

specificity.

Identification of the two isolates from 1966 is still pending.

FINAL DISCUSSION AND SUMMARY

It is hoped and expected that this preliminary survey will prepare some of the way for further work in elucidating the epidemiology of leptospirosis in Jamaica.

Till now it had been established that the disease does exist as a clinical entity and that wild rats in the Kingston area carry clinically important leptospires. The only serotypes isolated from patients and wild animals yet identified were <u>L's. icterohemorrhagiae</u> and <u>kremastos</u>. Several preliminary serologic surveys on incompletely defined human populations employing antigens of <u>L's. icterohemorrhagiae</u>, <u>canicola</u>, and <u>kremastos</u> have produced widely varying quantitative results. That leptospires infect many Jamaican domestic animals has been established serologically. Antibodies to a number of serogroups were demonstrated.

In the current study the nature of leptospirosis seen clinically in Jamaica was determined by a review of serologically and culturally demonstrated cases. The results showed that clinical leptospirosis on the island is similar to the severe disease picture in other parts of the world. A positive correlation between incidence of diagnosed infections and the quantity of rainfall was strongly suggested.

A preliminary serologic survey of three occupational groups -- abbatoire workers, sugar plantation personnel, and laboratory assistants and animal handlers -- and some of the animals in occupational contact with them was undertaken.

On the basis of the preliminary findings, a more extensive survey of persons working and living at the Caymanas Sugar Estates was begun, including serologic testing and a questionaire on occupational, social, and clinical histories. The prevalence of seropositivity found was considerably lower than might have been expected on the basis of certain previous serologic studies. A large proportion of those seropositive appeared by history to have had inapparent infections.

Antibodies to L. australis antigen, not previously noted in humans in Jamaica, were found in two subjects. The sera of these two had no activity against antigens of the leptospiral serotypes previously known to infect humans in Jamaica.

A number of domestic animals at slaughter were examined serologically with results similar to previous surveys. Antibodies to various serotypes were found.

Serologic and cultural efforts with wild animals trapped at the Caymanas Estates revealed serologic evidence of leptospiral infection in mongooses and the carrier state in both a rat and a mongoose. The isolates have yet to be identified as to species or pathogenicity.

The treatment of this disease is prevention. Clinically, supportive means are used. Antibiotic therapy has had only equivocal results in controlled studies (3).

In Jamaica more extensive epidemiologic studies would be helpful to assess the effects and importance of leptospiral infection in man and animals. Better defined human populations, both general and occupational, need to be surveyed serologically using a broader spectrum of antigens (including L. australis). Veterinary public health studies should attempt

to determine the economic importance of leptospirosis in domestic animals. A large survey of rats, mice, and mongooses around the island would be most helpful in identifying the more important carriers. Testing of water (by culture) from rivers, canals, and swamps in endemic regions, in conjunction with the search for carriers, may point the way toward effective control of this disease.

APPENDIX A

Procedure for Isolation of Leptospires from Animals

1. Specimens from aseptically removed kidneys are ground in a cold, sterile mortar with approximately 10 volumes of cold, sterile 0.02 M phosphate buffered saline solution (pH 7.2 - 7.4).
2. Further dilutions, 1:100, 1:1,000, 1:10,000 are made with the buffered solution.
3. One drop of each dilution is inoculated into individual screw-topped tubes with 5 cc of Fletcher's semi-solid medium (Difco) containing 8 - 10 per cent sterile rabbit serum (Difco Leptospiral Enrichment).
4. 0.1 ml of sterile 5-fluro-uracil solution (10 mg/ml) is added to each tube to reach a final concentration of 200 μg 5-fu per ml of medium (46).
5. Tubes are incubated in the dark at room temperature (25°C in Kingston).
6. Cultures are examined weekly under darkfield illumination for growth of leptospires for at least 30 days before being discarded as negative.

FORM 1.

Study No. .... Person No. ....

Name .... Date of collection ....

Age .... Sex M F

1. HOME SITUATION

A. Address ....
B. How long have you lived at present address ....
(C. If B is short Previous address ....)
(. ...)
D. Is house near: River Canal Swamp Sewer None
E. Do you have: Dog Pig Goat Cow Donkey Horse

11. OCCUPATIONAL SITUATION

A. Occupation ....
B. Place of business ....
C. Length of time on this or similar job ....
(D. If C is short, previous occupation ....)
E. Any more contact with: Donkey Horse Cow Goat Pig Rat Mongoose

111. CLINICAL HISTORY

A. Present complaint at clinic ....
B. Ever had: 1. Jaundice Date ....

2. "Hepatitis" " ....

3. "Yellow Fever" " ....

4. "Meningitis" " ....

5. Long high fever " ....

6. "Weil's disease" " ....

7. "Dengue fever" " ....

C. Ever stayed in hospital yes No Date ....
For what reason ....

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